





LEVAN PRODUCTION BY ACETIC ACID BACTERIA: SCREENING AND SELECTION **OF STRAINS**

KAVITHA ANGULURI (kavitha.anguluri@unimore.it)

Dept. Food Science and Technology, University of Modena and Reggio Emilia, Reggio Emilia, Italy

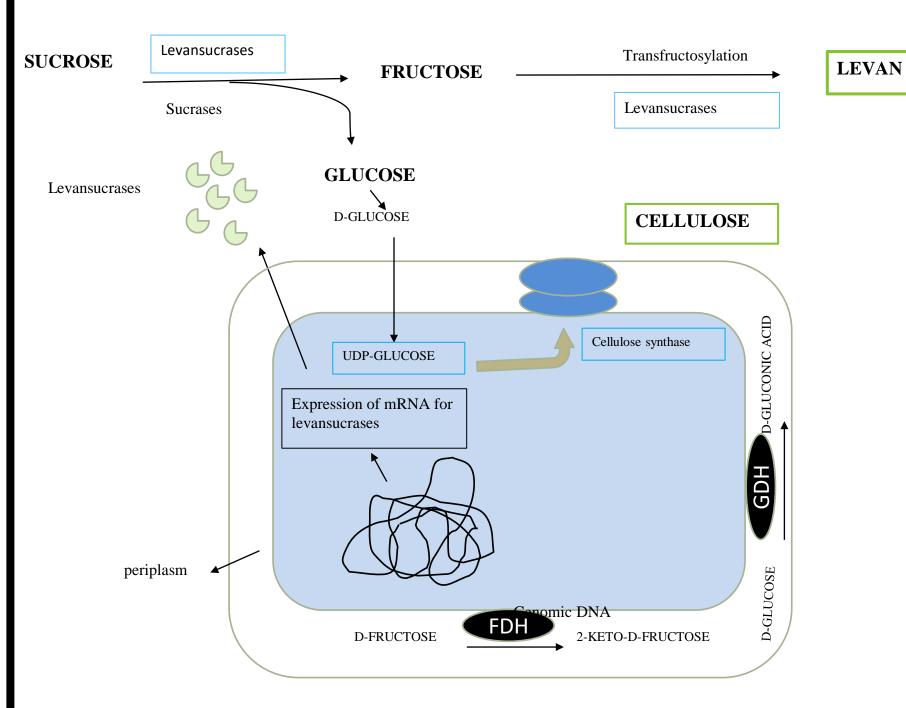
Tutor: Dr. MARIA GULLO

Background

Levan is a natural biopolymer produced from plants and microbes. Since microbial levan gained much attention due to its physicochemical properties, several strains belonging to gram-positive and gram-negative bacteria were tested to improve levan yield by applying suitable cultivation conditions. In this study, twelve acetic acid bacteria (AAB) strains were screened for their ability to produce levan by understanding the impact of environmental conditions in the medium. Strains belonging to same and different species showed varied pattern of metabolic behaviour and levan yield.

Introduction

- Levan or levan-type exopolysaccharides (LT-EPSs) are homopolysaccharide (HoPS) of the fructan type, consisting mostly of fructose monomers linked by β -(2,6) glycosidic bonds with possible β -(2,1) branches (Jakob et al. 2013).
- Metabolic pathways interfere with the production of levan such as transfructosyl activity, levansucrases, and hydrolysis activity.



Methods

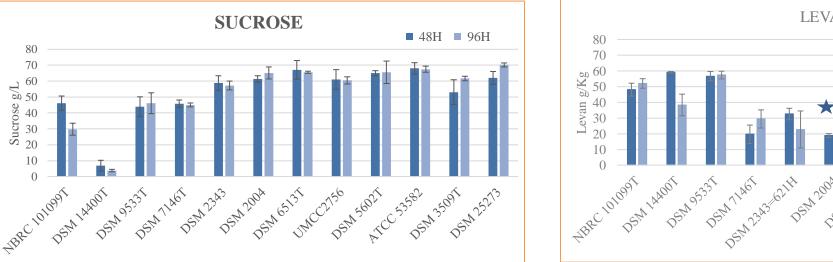
- ▶ 12 strains of AAB belonging to different species are tested for the levan production.
- ► HS media (peptone- 5 g/L, Yeast extract- 5 g/L, Na₂HPO₄-7.3 g/L, citric acid- 1.15 g/L, MgSO₄₋ 0.5 g/L) is used with glucose (20g/L) as preculture and glucose was replaced with sucrose (70g/L) to produce levan.

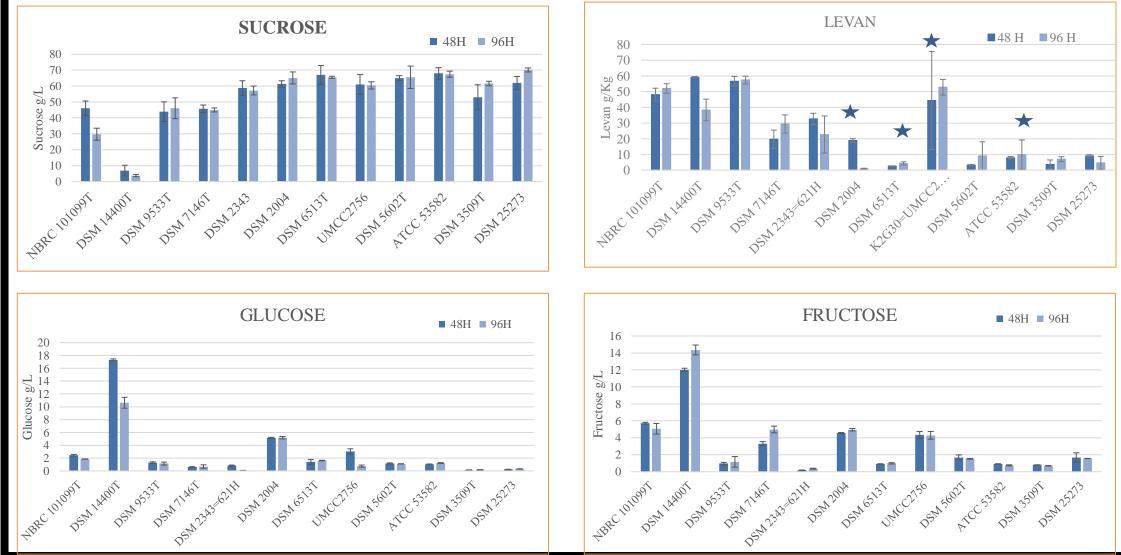
Environmental factors such as sucrose concentration, pH, temperature, and oxygen availability (aeration and agitation) are known to trigger the levan production (Öner et al. 2016).

Results

Influence of sucrose consumption on levan production:

- ▶ In this study, strains of *Gluconobacter* (G), *Acetobacter* (A), *Komagataeibacter* (K), Kozakia (Ko), and Neoasaia (N) genera (Table) were tested to produce levan at 70 g/L of sucrose as initial concentration.
- ► *K. baliensis* DSM 14400^T and *N. chiangmaiensis* NBRC 101099^T which are known as high producers of levan (Ua-Arak et al. 2016), consumed more sucrose (94% and 57% respectively) in 96 h compared to other strains.
- > Decrease in levan yield was observed in some strains which proved that levan is utilized by bacteria for survival under stress and starvation conditions (Öner et al., 2016).
- *Komagataeibacter xylinus* UMCC 2756 showed less consumption of sucrose and high levan yield. This strain also produced bacterial cellulose in glucose medium.





Experiment was carried out for 48h and 96h of incubation at 30°C and 140. Samples collected were analyzed for carbon sources consumption (sucrose, glucose, fructose), pH, gluconic acid, levan production by using Megazyme assay kit (K-SUFRG, K-GATE, K-FRUC, Megazyme, Ltd, Bray, Ireland).

Influence of substrate and acidity on levan yield

- Results obtained after 48h and 96h of cultivation showed a great variability among tested strains.
- Sucrose consumed by *K. baliensis* DSM 14400^T during 48 h was converted to glucose and sucrose by levansucrase which are further utilized for levan and gluconic acid production (Table).
- > pH was reduced in the medium to 3.11, which explains that bacteria under low pH stops producing levan and utilizing glucose for cell survival.
- *Gluconobacter* species consumed almost all the glucose in the medium, this explains their metabolism of glucose by 2 individual pathways (glucose oxidation and pentose phosphate pathway) (Ua-Arak et al. 2016).

STRAINS	рН		Gluconic acid	
	48h	96h	48h	96h
Neoasaia chiangmaiensis NBRC 101099 ^T	3.22	3.26	0.97	0.5
Kozakia baliensis DSM 14400T	3.11	2.88	10.12	7.36
Gluconobacter cerinus DSM 9533T	3.47	3.13	4.46	2.38
Gluconobacter frateurii DSM 7146T	4.04	2.97	1.79	1.32
Gluconobacter oxydans DSM 2343	4.76	4.58	0.37	0.07
Komagataeibacter xylinus DSM 2004	4.82	4.77	0.55	0
Komagataeibacter xylinus DSM 6513T	5.57	5.53	0.01	0.0006
Komagataeibacter xylinus K2G30	4.06	3.54	1.98	2.55
Komagataeibacter hansenii DSM 5602T	5.57	5.52	0.004	0.0005
Komagataeibacter hansenii ATCC 53582	5.58	5.55	0.004	0
Acetobacter pasteurianus DSM 3509T	5.5	5.5	0.96	0.71
Acetobacter pasteurianus AB0220	5.15	4.91	0.85	0.91

Future aspects

- Results showed great production variability among considered strains.
- It was also observed that both levan and bacterial cellulose were produced by the strain UMCC 2756 belonging to the species K. xylinus.
- > Although K. baliensis DSM 14400^T and N. chiangmaiensis NBRC 101099^T showed high levan yield, they have high variability in metabolic pattern of production > The utilization of different carbon sources lays a fundamental knowledge for further studies of optimizing strategies to improve levan yield

References

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